AMINO ACID, SUGAR AND ANTHOCYANIN FORMATION BY BEET SEEDLINGS AS AFFECTED BY DIFFERENT LEVELS OF K AND Ca OR NO_3 AND PO_4 IONS IN THE NUTRITIVE MEDIA

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ABSTRACT

In the presence of excess Ca, amino acid formation becomes low while carbohydrates are kept at relatively higher levels, thus increasing anthocyanin biosynthesis. In contrast, in the presence of excess K, NO_3 or PO_4 ions, amino acid formation becomes high and this leads to low carbohydrate levels in the seedlings thus decreasing anthocyanin formation.

INTRODUCTION

The relation between total sugar contents and uptake of nutrient elements by plants has been studied by many workers. Humphries (1951-1952) found that the uptake of N, P and K elements by barley and pea seedlings, cultivated in nutrient solutions having different concentrations of these elements, was increased by the increase of total sugar contents of plants. The same author (1956) showed that the positive regression of nutrient uptake was due to the reducing sugar fraction. He concluded that the conditions inducing high reducing sugars also induce high rate of uptake of elements and vice-versa. Nosseir (1972) using pea seedlings grown in nutrient solutions having differing K and Ca levels found that there was a fair connection between reducing sugars and sucrose on one hand and uptake of K, N and P elements on the other hand. Under such conditions, sugars may be the parent substance for the formation of a chemical compound capable of combining with K, P or N as suggested by Humphries (1952).

Stiles (1936) stated that accumulation of Ca in beet plants was accompanied by accumulation of carbohydrates in the absence of N. The function of Ca in this case was to neutralize the acids derived from carbohydrate breakdown during respiration. In the presence of N, carbohydrate breakdown derivatives will be utilized in the formation of organic nitrogenous compounds and this leads to the disappearance of carbo-

hydrate derivatives inside the tissues. Steward et al (1940), Said & El Shishiny (1949) and Nosseir (1972) using storage tissues or intact seedlings found that Ca ions inhibit absorption and assimilation of nitrate ions as well as protein synthesis and respiration leading to accumulation of carbohydrate derivatives inside the tissues. In contrast, the same authors found that K ions produced diverse processes, i.e. activation of uptake and assimilation of nitrate ions with simultaneous increase in respiration and protein synthesis leading to disappearance of carbohydrate derivatives from the tissues. In such a case, carbohydrate derivatives were used as energy sources and as C skeletons for the organic nitrogenous compounds which were formed.

Gregory & Sen (1937) and Yemm & Willis (1956) found that amino acid synthesis in leaves and roots of barley seedlings was dependent on their sugar content and that a considerable loss of reducing sugar in the tissues was accompanied by a marked increase of amino acids, mainly glutamic acid. The biosynthesis of anthocyanin pigment in intact seedlings or excised parts is recognized to be affected by many factors, particularly light and carbohydrate. The anthocyanin molecule on hydrolysis by dilute HCl gives 2 molecules of glucose and an anthocyanidin molecule (Stiles, 1936). The purpose of this study is to determine the total soluble sugars, total amino acids and anthocyanin contents of beet seedlings as affected by the presence of different K:Ca or N:P ratios in the culture media by varying the concentration of one of these ions with all the other ions remaining constant.

MATERIAL AND METHODS

Seed balls of *Beta vulgaris* "Bettrave" imported from France, were provided by the Egyptian Ministry of Agriculture in Cairo. Owing to the fact that the coats of these seed balls accumulate germination inhibiting substances and because of the strong solubility of these substances in water (El-Shishiny & Thoday, 1953), it was necessary to rinse the balls prior to germination.

Seven lots of beet seed balls, each lot composed of 50 seeds, were washed and leached with running distilled water for 8 hr to eliminate the germination

inhibitory substances. They were then rinsed in 70% ethyl alcohol for surface sterilization (Hatata et al, 1979), washed several times with sterile distilled water, and then distributed in large petri dishes, each having two filter papers moistened with 20 ml sterile distilled water. Each dish containing 50 seeds was transferred to a lightened thermostatically-controlled incubator adjusted to constant temperature of 25°C and constant light intensity of 6000 lux (Nosseir, 1968). At he 10th day of growth, seedlings were divided into 10 samples. Each sample was composed of 50 seedlings. The seedlings were then transferred into sterile 1 liter culture vessels, each fitted with a sintered glass bubbler and containing 500 ml of distilled water or 500 ml of experimental solution according to the following design:

1) normal Hoagland solution designated $6K^+:5Ca^{++}$;
2) normal Hoagland solution plus 0.002M KCl designated $8K^+:5Ca^{++}$;
3) normal Hoagland solution plus 0.004M KCl designated $10K^+:5Ca^{++}$;
4) normal Hoagland solution plus 0.002M $CaCl_2$ designated $6K^+:7Ca^{++}$;
5) normal Hoagland solution plus 0.004M $CaCl_2$ designated $6K^+:9Ca^{++}$;
6) normal Hoagland solution designated 15N:1P;
7) normal Hoagland solution plus 0.002M $NaNO_3$ designated 17N:1P;
8) normal Hoagland solution plus 0.005M $NaNO_3$ designated 20N:1P;
9) normal Hoagland solution plus 0.002M NaH_2PO_4 designated 15N:3P;
10) normal Hoagland solution plus 0.004M NaH_2PO_4 designated 15N:5P.

Before and during the period of plant culturing the pH was adjusted to 5.8-6.0. Sterile compressed air was allowed to pass through the culture vessels at a constant rate till the end of the experiment (7 days).

The seedlings were then taken out, washed with distilled water, dried gently, and weighed. Dry weights were obtained by keeping the samples in a drying oven at 105°C until the weight became constant. The dry matter was ground to fine powder and then used for the estimation of total soluble sugars and total free amino acids.

Extraction, separation and determination of soluble sugars and total free amino acids were done using the methods adopted by Nosseir (1968). A known weight (100 mgm) together with a water-alcohol mixture (30:70 by volume) were transferred into a conical flask fitted with an air condenser. The flask was then immersed in a boiling water bath, cooled and agitated for 6 minutes using a shaker. The suspension was then filtered and the filtrate was evaporated to a final volume of 4 ml. The sugars were then separated from the amino acids in the filtrate using the universal resin column where amino acids are adsorbed on the surface of the resin particles while sugars and organic acids flow down freely.

Amino acids were then eluted by 10% ammonia and the eluate was received in a crucible and evaporated to dryness. The total sugar content was determined calorimetrically using the anthrone method, while amino acid content was determined using the ninhydrin method (Nosseir, 1968). For anthocyanin determination, the seedlings of each sample were taken out from their respective solutions, washed with distilled water and treated with 100cc of 1% HCl (Pecket, 1965) and left for 48 hours for anthocyanin extraction. The extract was decanted and the seedlings were washed 3 times each with 10cc of 1% HCl and the washing was added to the extract, which was then completed to 200 ml by adding distilled water.

The specific density of anthocyanin was estimated by using the spectronic 20 colorimeter at 530mμ.

RESULTS

Amino acid, sugar and anthocyanin formation in beet seed-lings as affected by differing levels of K and Ca in the nutritive media. The total soluble sugar and the total free amino acid contents of beet seedlings cultured in nutrient media having different relative concentrations of K and Ca ions are shown in Fig (1).

Figure (1) reveals that control seedlings cultured in 6K:5Ca solution contained 10.8 mgm sugars and 11.0 mgm amino acids. Increasing the concentration of the K ions in the culture media by 33% and keeping the concentration of all other ions constant, exerted an increase of 34.5% in amino acid content over that of

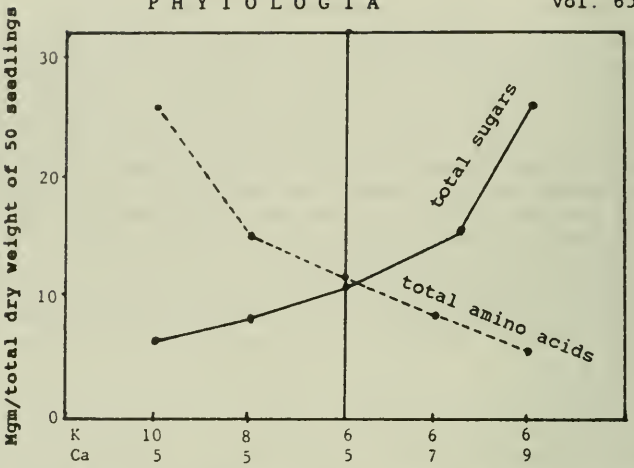

Fig (1): Total sugar and amino acid contents of the total dry weight of 50 beet seedlings cultured for 7 days in 500 ml of distilled water or Hoagland solution alone and in combination with different K or Ca levels in the culture media. Means of 4 replicate samples in mgm per dry weight of 50 seedlings.

control seedlings cultured in 6K:5Ca solution with a simultaneous decrease of 25% in sugar content.

Further increase in K concentration in the culture media by 66% under similar conditions, further increases amino acid content to 132% of control. While amino acid contents increase, Fig (1) reveals a simultaneous decrease of 44% in sugar content.

On the other hand, increasing the concentration of Ca ions in the culture media of beet seedlings produced varied effects. Increasing the concentration of Ca ions in the culture media by 40% and 80% over controls (6K:5CA), resulted in increases of 37.9% and 137.0% in total sugar contents with simultaneous decreases of 26.3% and 54.5% in total amino acid content below those of the controls respectively.

The above results clearly indicate that the presence of excess K ions in the culture media of beet seedlings exerts a stimulating effect on the formation

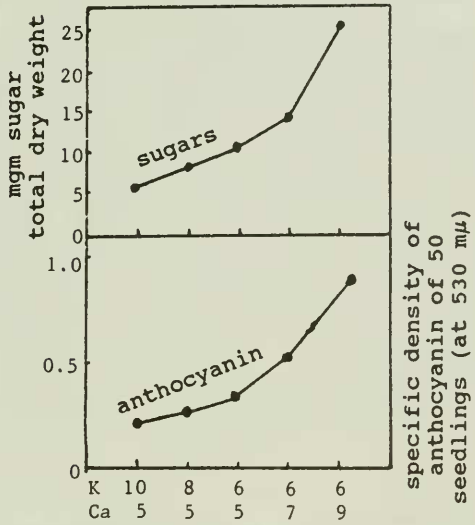

Fig. (2): Total sugar and specific density of anthocyanin content of 50 beet seedlings aerated for 7 days in 500 ml of distilled water or Hoagland solution alone and in combina-tion with different levels of K or Ca in the culture media. Mean values of 4 replicate samples for sugars and mean values of duplicate samples for anthocyanin contents.

of free soluble amino acids in the tissues, while the presence of excess Ca ions exerts retarding effects. In both cases the synthesis of amino acids appears to be at the expense of the total soluble sugar since reciprocal relationships were obtained under different nutritional conditions as clearly illustrated in Fig (1). The data concerning anthocyanin content of beet seedlings cultured in different culture media are given in Fig (2). These data represent the specific density of anthocyanin content of 50 seedlings estimated in mμ, and the relation between total sugar and the corresponding anthocyanin content of the seedlings, as well as the changes due to the presence of excess K or Ca ions in the culture media as compared with controls (6K:5Ca). Under control conditions (6K:5Ca), corresponding values for anthocyanin content is 0.35 mμ and sugar content is 10.8 mgm. Increasing the content of K ions in the culture media by 33% and 66% over that of

control seedlings, resulted in decreases of specific density of anthocyanin contents of 50 beet seedlings by 20% and 40% respectively, with simultaneous decreases in sugar content by 25% and 44.4%. But increasing the concentration of Ca ions in the culture media by 40% and 80% over that of control seedlings, resulted in increases of specific density of anthocyanin content by 51.4% and 151.4% respectively, with simultaneous increases in their corresponding sugar contents by 38.0% and 137.0%. Thus the presence of excess K in the culture media of beet seedlings exerted similar depressing effects on the formation of both anthocyanin and sugar contents, while the presence of excess Ca exerted stimulating effects on the formation of both anthocyanin and soluble sugars. In other words, parallel relationships exist between total soluble sugars and anthocyanin content in beet seedlings.

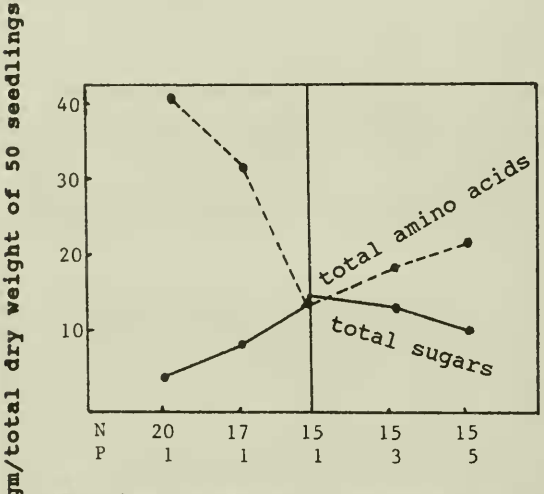

Fig (3): Total sugar and amino acid contents of the total dry weight of 50 beet seedlings cultured for 7 days in 500 ml distilled water or Hoagland solution alone and in combination with differing NO_3 and PO_4 levels. Means of 4 replicates in mgms per total dry weight of 50 seedlings.

Amino acid, sugar and anthocyanin formation in beet seedlings as affected by differing levels of NO_3 and PO_4 ions in the nutritive media. The total soluble sugars and the total free amino acid contents of beet seedlings cultured in nutrient media having different concentrations of NO_3 and PO_4 ions are shown in Fig

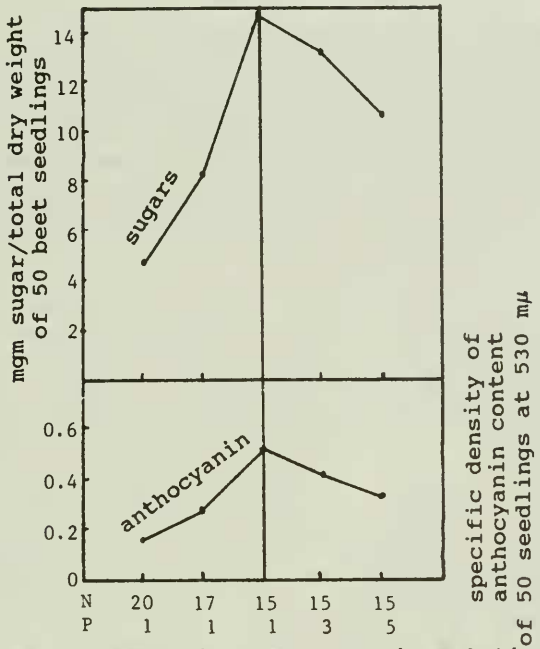

Fig. (4): Total sugars and specific density of anthocyanin content of 50 beet seedlings aerated for 7 days in 500 ml distilled water or Hoagland solution alone or in combination with differing PO_4 or NO_3 levels. Mean values of 4 replicate samples for sugars and mean values for duplicate samples for anthocyanin contents.

(3). This figure reveals that the control seedlings cultured in 15N:1P solution contained 14.8 mgm sugar and 13.3 mgm amino acids. Increasing the concentration of NO_3 ions by 13% and 23% or increasing the PO_4 ions by 200% and 400% over the culture medium (while keeping the concentration of all other ions constant) caused increases of 138.3%, and 203.0% or 36.0% and 60.9% respectively in the amino acid contents. Under similar conditions there were corresponding decreases of 45.3% and 68.9% or 11.5% and 28.4% in the sugar contents below that of the control seedlings.

From the above results, it becomes obvious that the presence of excess NO_3 or PO_4 ions in the culture

media exerted stimulating effects on the formation of free soluble amino acids in the tissues at the expense of their total soluble sugars, since reciprocal relationships between these two fractions were obtained for different culture media as clearly shown in Fig (3). But the magnitude of changes due to the presence of NO_3 ions in the culture media were higher than the corresponding changes due to the presence of PO_4 ions, although the concentrations of the latter ions were much higher than the former ions.

The data concerning anthocyanin contents of beet seedlings cultured in media having differing concentrations of NO_3 or PO_4 ions are presented in Fig (4). The anthocyanin content of 50 beet seedlings cultured in control solution (15N:1P) was 0.510 mμ corresponding to 14.8 mgm sugars per total dry weight. Increasing the concentration of NO3 ions by 13% and 26% or PO4 ions by 200% and 400%, while keeping the concentrations of all other ions constant, caused decreases of 43.5% and 70.5% or 13.7% and 33.3% respectively in the anthocyanin contents below that of control seedlings. In the meantime corresponding decreases of 45.3% and 68.9% or 11.5% and 28.4% in the sugar contents below that of controls were also obtained.

Keeping in mind the release of additional glucose as a result of anthocyanin hydrolysis during estimation of total soluble sugars by the anthrone method, it can be concluded that biosynthesis of anthocyanin had taken place at the expense of soluble sugars since a parallel relationship exists between total soluble sugar and anthocyanin content, whether the seedlings were cultured in media differing in either PO_4 or NO_3 content.

The decrease in both sugar and anthocyanin contents of seedlings cultured in media having excess nitrate ions was much more pronounced than the corresponding decrease in seedlings cultured in media with excess phosphate, although the concentrations of the nitrate ions were much lower than those of the phosphate ions.

DISCUSSION

Effects of K and Ca levels:

Stiles (1936) stated that in the presence of N in the culture media of beet plants, the carbohydrate derivatives are utilized in the formation of organic nitrogenous compounds and this leads to their disappearance. From Fig (1) it becomes apparent that increasing the concentration of K ions in the nutritive media and keeping the concentrations of all other ions constant, a progressive increase in nitrate assimilation results accompanied by progressive increases in amino acid contents but progressive decreases in total sugar contents. In contrast, increasing the concentration of Ca ions in the nutritive media yields varied results.

The results depicted in Fig (2) clearly illustrate that with increments of K or Ca concentrations, there is always a parallel relationship between the total soluble sugars and anthocyanin content.

Effects of NO_3 and PO_4 levels:

Increasing the concentration of NO_3 and PO_4 ions and keeping the concentration of the other ions constant, resulted in progressive increases in nitrate assimilation accompanied by progressive increases in amino acid contents but with progressive increases in total soluble sugars and anthocyanin content (Fig (3). But the magnitude of changes due to the presence of nitrate concentrations were much greater than those due to the presence of altered phosphate (although the concentrations of phosphate ions were much greater than nitrate ions).

The parallel effects of nitrate or phosphate on the above mentioned processes in beet seedlings repeat the correlation between N and P metabolism previously found by Cole et al (1963) in corn seedlings and by Nosseir (1972) in pea seedlings.

Under all the nutritional conditions used in the present study, there was a reciprocal relationship between amino acid contents of the seedlings on the one hand and their total soluble sugar contents on the other hand, indicating the dependence of amino acid formation on sugars. Similarly, Gregory & Sen (1937) found that amino acid synthesis in barley leaves takes place at the expense of organic acid derived from sugars. Again, Yemm & Willis (1956) found that the synthesis of amino acids in barley roots was dependent

on their sugar contents and that a considerable loss of the reducing sugars of these tissues was accompanied by a marked increase of amino acids, mainly glutamic acid. Nosseir & Hathout (1970) and Nosseir (1972) established that the amino acid synthesis in sweet potato tuber discs and intact pea seedlings respectively, took place at the expense of total sugar contents of the tissues. In the present study there was a parallel relationship between total soluble sugars and anthocyanin content of the seedlings; this indicated a dependence of anthocyanin biosynthesis on sugars. Because of the low rates of nitrate assimilation and amino acid formation in the presence of excess Ca ions, carbohydrates might have been preserved and kept at relatively higher levels, thus increasing anthocyanin formation. In contrast, when the rate of nitrate assimilation and amino acid formation in the presence of excess K, PO_4 or NO_3 ions were increased, carbohydrate levels in the tissues became lower, thus decreasing anthocyanin formation. This fact has already been stressed by Mohammed & Collins (1978), Troyer (1964) and Malaviya et al (1966), who found that glucose or sucrose supplied to intact seedlings or excised hypocotyls increased their anthocyanin contents.

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